PATENT SPECIFICATION

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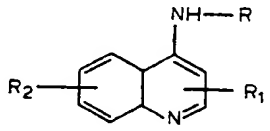
(54) IMPROVEMENTS IN OR RELATING TO NEW 4-AMINO-QUINOLINE DERIVATIVES PROCESS FOR THEIR PREPARATION AND THERAPEUTIC APPLICATIONS THEREOF

(71) We, SERDEX, Societe d'Etudes, de Recherches, de Diffusion et d'Exploitation, a French Body Corporate, residing at Tour Beau 20 Rue Jean-Jaures, 92800 Puteaux, France, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to new 4-amino-quinoline derivatives having the general formula:



(I)

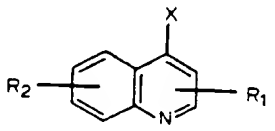
in which R is a straight- or branched-chain alkyl group having at least 10 carbon atoms and R_1 and R_2, which may be the same or different, each represent typically hydrogen, halogen, an alkyl, aryl, hydroxy, ether, thioether, amino, alkylamino, dialkylamino, nitro or trifluoromethyl group, and the acid addition salts of said derivatives.

R_1 and R_2 are preferably each hydrogen, halogen, or a lower alkyl, phenyl, hydroxy, lower alkoxy, loweralkyl-thio, lowerdialkylamino, nitro or trifluoromethyl group, By "lower alkyl" or "lower alkoxy" are meant groups of this type containing 1—6 carbon atoms.

The acids useful to convert the compounds (I) to salt form are preferably thereapeutically acceptable acids.

Indeed, it was found that the compounds (I) and their salts exhibit useful therapeutic properties, particularly antamoebic, antibacterial and antifungal properties which make them applicable in human and veterinary medicine.

The compounds (I) may be prepared by reacting, preferably at elevated temperature, in the presence or in the absence of solvent, a quinoline of the formula:



(II)

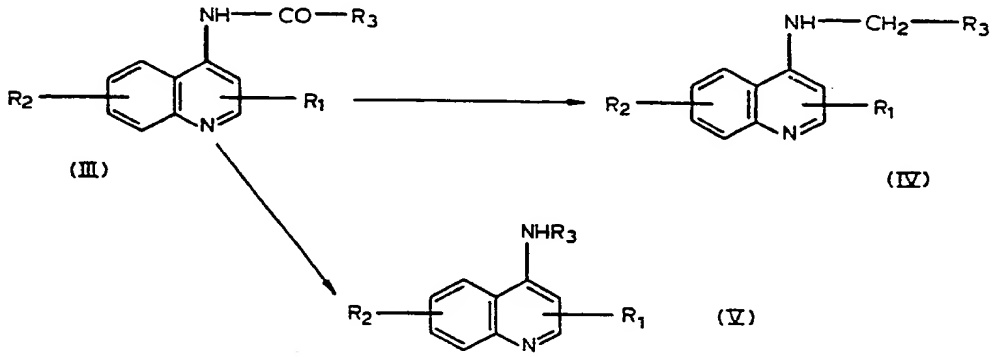
in which X represents a cleavable grouping of the halogen (chlorine, bromine, iodine), HO, aryloxy (Ar—O—), alkyl or aryl thioether, alkyl or arylsulfonyl type, with a primary amine NH_2R.

Useful solvents include alcohols, nitro derivatives (e.g., nitromethane), acetonitrile, or phenol, or mixtures thereof.

The reaction is generally conducted by reacting one mole of (II) with 2.2 moles of amine NH_2R, preferably at elevated temperature. The reaction may also be carried out by using simply one mole of amine NH_2R, provided it is conducted in the presence of a tertiary amine such as triethylamine capable of binding the XH formed and which does not react with compound (II). This technique is particularly useful when X is halogen, the resulting triethylamine salt being water soluble, whereas the salt of amine NH_2R is not.

It should be noted that after the compound (I) is obtained it may be converted to another compound (I) by chemical modification of substituents R_1 and/or R_2. Thus, a hydroxy substituent may be converted to a halogen substituent by action of the corresponding phosphorus oxyhalide. Similarly, an alkoxy substituent may be cleaved to a hydroxy group, and a halogen substituent may be converted to hydrogen by catalytic hydrogenation.

As a modification, it is also possible to reduce an amide (III), in which R_3 is an alkyl chain having at least 9 carbon atoms, to an amine (IV), using lithium aluminium hydride in a solvent such as ether, or dioxan.



It is obvious that this method cannot be used when R_1 and R_2 are also reduced by the reagent.

Secondary amine (V) is also obtained from amide (III) by oxidation with a hypochlorite or a hypobromite.

This constitutes a second modification for the preparation of compounds (I), provided R_3 contains at least 10 carbon atoms.

The salts are prepared by reacting an inorganic or organic acid with amine (I), (IV) or (V) dissolved in a suitable solvent which is then evaporated off, after which the salt is purified by crystallization.

The following non-limiting examples are given to illustrate the invention.

Example 1.

4-n-Dodecylamino-quinoline

4-Chloro-quinoline (1 mole) is heated for 3 days, at 120°C, with n-dodecylamine (2.2 moles). The resulting solid is taken up into hot water, in the presence of excess sodium hydroxide. The oil released is decanted off and is then extracted with a solvent (e.g. $CHCl_3$, or C_6H_6). After removal of the solvent, the primary amine is recovered by distillation *in vacuo*. The residue is crystallized from heptane. The product melts at 80°C (Yield: 86%).

Example 2.

2,8-Dimethyl-4-n-decylamino-quinoline

4-Chloro-2,8-dimethyl-quinoline (1 mole) and n-decylamine (2.2 moles) are heated at 120°C for 9 days. The bases are released as described above. The secondary amine is isolated either by distillation $b.p._{0.9}$ = 224—225°C or by crystallization from heptane; m.p. = 57°C (Yield: 72%). Monohydrochloride, monohydrate: m.p. = 98—99°C.

Example 3.

2,8-Dimethyl-4-n-decylamino-quinoline

4-Chloro-2,8-dimethyl-quinoline (1 mole) and n-decylamine (1 mole) are

heated at 120—130°C with triethylamine (1.2 mole). The reaction mixture is taken up into water, in the presence of a solvent such as chloroform, or benzene to remove the resulting triethylamine hydrochloride and the excess tertiary amine. After removing the solvent, the secondary amine is crystallized or distilled (Yield: 70%).

Example 4.

4-n-Decylamino-quinoline

4-Methylsulfonyl-quinoline (II, $X = CH_3SO_2$—, $R_1 = R_2 = H$) (1 mole) and n-decylamine (2.2 moles) are heated at 150—170°C for 48 hours. The secondary amine is isolated as previously described; m.p. = 79°C (heptane). (Yield: 60%).

Example 5.

2,8-Dimethyl-4-n-dodecylamino-quinoline

4 Phenoxy-2,8-dimethyl-quinoline (1 mole) and n-dodecylamine (1.1 mole) are heated at 120°C for 9 days. The reaction mixture is taken up into water made alkaline with excess sodium hydroxide. The secondary amine is crystallized from heptane; m.p. 64°C (Yield: 72%).

Example 6.

7-Chloro-4-n-decylamino-quinoline

4,7-Dichloroquinoline (1 mole) and decylamine (2.2 moles) are heated at 120°C for 4 days in the presence of phenol (1.2 mole). The secondary amine is isolated as previously described; m.p. = 99°C (ethyl acetate) (Yield: 88%).

Example 7.

2-Hydroxy-4-n-decylamino-quinoline

2,4-Dihydroxy-quinoline (1 mole) and n-decylamine (3 moles) are refluxed for 24 hours. After distillation of the excess primary amine, the secondary amine is crystallized from ethanol; m.p. = 152°C (Yield: 57%).

Example 8.

2-Chloro-4-n-decylamino quinoline

The preceding hydroxyl derivative (1 mole) and phosphorus oxychloride (12 moles) are refluxed for 44 hours. After removal of the excess chlorinating reagent, the reaction mixture is taken up into ice made alkaline with an alkali metal carbonate, the chloro derivative is extracted with chloroform. The solvent is removed and the product is then crystallized from octane; m.p. = 67°C (Yield: 60%).

Example 9.

(a) *Ethyl 2-(2-isopropyl-phenylamino)-crotonate*

o-Isopropylaniline (1 mole) is mixed with ethyl acetoacetate (1.05 mole) in the presence of 3 drops hydrochloric or acetic acid. After 48 hours, the water formed is separated by decantation. The crotonate is distilled under reduced pressure. $b.p._{0.7}$ = 127—129°C. Yield: 66%.

The water formed may also be removed by operating *in vacuo*, over sulfuric acid, or it may be entrained by azeotropic distillation in the presence of benzene.

(b) *2-Methyl-8-isopropyl-4-hydroxy-quinoline*

The above crude crotonate (1 mole) is gradually added to 1 litre boiling diphenyl oxide or to 1 litre paraffin oil heated at 260°C, allowing the alcohol formed to distil off. After cooling, the resulting solid is suction filtered, washed with a solvent (such as trichloroethylene).

It is recrystallized from toluene. M.p. = 173—174°C (Yield: 80%).

(c) *4-Chloro-2-methyl-8-isopropyl-quinoline*

The above hydroxyl derivative (1 mole), dried at 105°C, is added to phosphorus oxychloride (750 ml) heated at 70—80°C. The temperature is maintained at 80°C during 3 hours. After removing the phosphorus oxychloride *in vacuo*, the resulting material is poured over ice in the presence of chloroform. It is then neutralized with an alkali metal carbonate, sodium hydroxide or ammonia. The chloroform solution is separated. After removal of the solvent, the quinoline derivative is isolated; $b.p._{0.5}$ = 105°C (Yield: 93%).

(d) *8-Isopropyl-4-n-decylamino-quinaldine*

4-Chloro-8-isopropyl-quinaldine (1 mole) and n-decylamine (2.2 moles) are heated at 120°C during 19 days. The amines are released by action of dilute sodium hydroxide and the insoluble oil is distilled. The secondary amine is collected; $b.p._{0.5}$ = 211°C 68%).

Example 10.

4-n-Tetradecylamino-quinoline

4-Myristoylamino-quinoline (III, $R_3 = C_{13}H_{27}$, $R_1 = R_2 = H$) (0.5 mole) is reduced with lithium aluminum hydride (0.5 mole) in the presence of 2 litres boiling ether or of dioxan at 40—50°C; the excess reagent is destroyed by addition of dilute sodium hydroxide, at about 0°C. The insoluble inorganic materials are removed and washed with ether. The solvent is distilled off; the crude secondary amine is purified by crystallization; m.p. 81°C (heptane). Yield: 70%.

Example 11.

2-Methyl-4-n-decylamino-quinoline

4-Undecanoylamino-quinaldine (1 mole) is added, with stirring to a cooled mixture (0°C) of bromine or chlorine (1.02 mole) and potassium or sodium hydroxide (5.5 moles) in water (8 litres). The reaction mixture is gradually heated to 70—75°C and is maintained at that temperature for 45—60 minutes. The amine is extracted with a solvent such as benzene or chloroform, and purified as previously described.

Example 12.

3-Nitro-4-n-decylamino-quinoline

The above derivative is obtained by reacting 3-nitro-4-chloro-quinoline (0.5 mole) with n-decylamine (1.1 mole) in the presence of nitromethane (1 litre) at 60°C, for 24 hours, with stirring. After removal of the nitromethane by distillation, the bases released with sodium hydroxide are extracted with chloroform. The chloroform is distilled off at atmospheric pressure and the primary amine is then distilled off *in vacuo*. The 3-nitro-4-n-decylamino-quinoline residue is purified by crystallization from petroleum ether; m.p. = 53—54°C (Yield: 76%).

Example 13.

8-Methoxy-4-decylamino-quinaldine

4-Chloro-8-methoxy-quinaldine (1 mole) and decylamine (2.2 moles) are heated at a temperature of 90°C in the presence or in the absence of methanol (800 ml). The derivative is isolated as previously described. $b.p._{0.2}$ = 226°C; m.p. = 117°C (acetone-water) (Yield: 76%).

Example 14.

8-Methoxy-4-decylamino-quinaldine

4-Chloro-8-methoxy-quinaldine (1 mole) is treated with decyl amine (2.2 moles) and phenol (1.2 mole) dissolved in methanol (800 ml) at 90°C. After removal of the alcohol, the material is made alkaline with excess sodium hydroxide, the amines are solvent extracted (e.g. chloroform), and the secondary amine is separated by distillation.

Example 15.

8-Hydroxy-4-decylamino-quinaldine

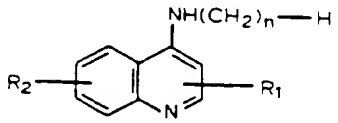
The preceding derivative is heated to boiling with 4 times its weight of pyridine hydrochloride, for 30 minutes. The reaction mixture is taken up into water and the insoluble hydrochloride is isolated by suction filtering. It is suspended in chloroform and excess ammonia (50% concentration) containing 1—2% sodium dithionite is added thereto. The chloroform solution is then evaporated *in vacuo*, to give 8-hydroxy-4-decylamino-quinaldine; m.p. 84.5°C (nitromethane) (Yield: 83%).

Example 16.

4-n-Dodecylamino-quinoline

The compound is obtained, according to a modification, from 7-chloro-4-n-dodecylamino-quinoline (0.02 mole). The latter, dissolved in methanol containing 1.35 g potassium hydroxide, is hydrogenated in the presence of Raney nickel until 0.02 mole hydrogen has been taken up. After removal of the catalyst and the alcohol, the amine is solvent extracted and crystallized from hexane.

Table 1 below indicates the constants of the compounds obtained according to the above examples and of other compounds (I) obtained in a similar manner. To identify the compounds, reference will be made to formula (Ibis) in which R is developed to $(CH_2)_nH$:



(Ibis)

On the other hand, the positions of substituents R_1 and R_2 on the quinoline nucleus are numbered according to the conventional nomenclature.

TABLE 1.

n	R_1	R_2	M.p. (°C) or b.p. (°C)	CODE
10	H	H	79	RC61
12	H	H	80	RC2
14	H	H	81	RC3
16	H	H	85	RC4
18	H	H	84	RC5
10	2—CH_3	H	76.5	RC410
12	2—CH_3	H	69.5	RC57
14	2—CH_3	H	71	RC58
16	2—CH_3	H	75	RC59
18	2—CH_3	H	76	RC60
10	3—CH_3	H	$b.p._{0.3}$ = 198—201	RC16
10	H	6—CH_3	87.5	RC14
10	H	7—CH_3	102	RC17
10	H	8—CH_3	64	RC10
10	H	8—C_2H_5	47	RC47
10	H	8—i—C_3H_7	46.5	RC38
10	2—CH_3	5—CH_3	77	RC31
10	2—CH_3	6—CH_3	52	RC8
10	2—CH_3	8—CH_3	57	RC284
12	2—CH_3	8—CH_3	64	RC7
10	2—CH_3	8—C_2H_5	45	RC46
10	2—CH_3	8—i—C_3H_7	$b.p._{0.5}$ = 211	RC12
10	2—Cl	H	67	RC21
10	H	6—Cl	97	RC18
10	H	6—F	71	RC44
10	H	7—Cl	99.5	RC19

TABLE 1 (continued)

n	R_1	R_2	M.p. (°C) or b.p. (°C)	CODE
12	H	7—Cl	93	RC53
14	H	7—Cl	88	RC54
16	H	7—Cl	90	RC55
18	H	7—Cl	89	RC56
10	H	8—Cl	80	RC20
10	H	8—F	70	RC45
10	2—CH_3	6—Cl	89	RC22
10	2—CH_3	6—F	80	RC48
10	2—CH_3	7—Cl	102	RC23
10	2—CH_3	8—Cl	91	RC24
10	2—CH_3	8—F	76	RC49
10	H	6—OCH_3	89	RC25
10	H	7—OCH_3	90	RC26
10	H	8—OCH_3	123	RC27
10	2—CH_3	6—OCH_3	76.5	RC28
10	2—CH_3	7—OCH_3	77	RC29
10	2—CH_3	8—OCH_3	117	RC30
10	2—CH_3	8—SCH_3	99	RC50
10	3—NO_2	H	53—54	RC32
10	H	6—NO_2	120	RC33
10	H	7—NO_2	126	RC34
10	H	8—NO_2	82	RC35
10	2—OH	H	152	RC51
10	2—CH_3	8—OH	84.5	RC62
10	2—CF_3	H	95	RC41
10	H	7—CF_3	85	RC40
10	H	8—CF_3	81	RC37
10	2—CH_3	8—$N(CH_3)_2$	75—76	RC52
10	2—C_6H_5	H	71	RC65
10	H	5—OCH_3	54—55	RC66

As previously mentioned, the compounds (I) and their salts exhibit an amoebicidal activity, an antibacterial activity, particularly against gram-positive bacteria, and an antifungal activity, particularly against candida albicans.

The amoebicidal activity has been evaluated *in vitro* on cultures of *Entamoeba histolytica* and also *in vivo* in experimental amebiasis of young rats infested with the same parasite.

A. *In vitro* tests

Said tests were conducted with cultures of *Entamoeba histolytica* of human origin, maintained on PAVLOVA-JONES monophase medium (JONES W.R., Experim.Parasit., 1952, *1*, p.118—128) according to two different techniques:

(1) *inhibition at the beginning of the cultures*

The test involves the determination of the smallest amount of material which, added to the culture medium prior to seeding, completely inhibits the growth of the amebae after a contact time of 72 hours in an oven at 37°C.

(2) *Lethal action on a two-day culture*

In this series of tests, the smallest amount of material which, added to a fully growing culture (2-day culture), is capable of killing all the amebae after 48 hours in an oven at 37°C is determined.

Some of the results obtained are summarized in following Table 2. Columns 1, 2 and 3 indicate the reference of the compound, the inhibition at the beginning of cultivation and the lethal action, respectively, in terms of microgrammes per ml.

TABLE 2.

Reference	Inhibition at the beginning of the culture	Lethal action
RC2	0.5	25
RC12	0.25—0.5	2.5—3.1
RC17	0.5	10
RC19	0.5—1.25	5—10
RC25	0.5—1	5
RC30	0.062—0.125	1.25
RC33	0.5	12.5
RC34	1	5
RC61	0.125—0.5	5—25
RC284	0.125—0.31	0.62—5
RC284.HCl	0.10—0.5	1.25—2.5

B. *In vivo tests*

The in vivo tests were conducted according to a technique closely related to that described by JONES (JONES W.R., Brit. J. Pharmacol., 1967, *2*, p. 217—220) and discussed by R. CAVIER & J. CENAC (Bull. Soc. Pat. Exot., 1972, *65*, p. 399—404).

The tests animals used are young rats, immediately after weaning, weighing 25—35 g.

After aseptic laparotomy, under nembutal-induced anesthesia, (1% solution in sterile distilled water; intraperitoneal injection of 0.50 ml per 100 g of body weight of the animal) 0.5 ml of a culture of *E. histolytica* on di-phase medium (Pasteur Institute) containing about 200.000 pathogenic amebae is inoculated in the cecum. Treatment begins 24 hours after infestation and comprises administering the test material suspended in a mixture of equal parts of water and gum syrup, by the oral route, once daily during four days.

Autopsy is carried out 48 hours after the last ingestion. The cecum is examined macroscopically; its contents and the material obtained on scraping the mucosa of the cecum are examined under the microscope.

In each series of experiments, a number of animals are not given any treatment and are used as reference of the infestation.

The results are expressed according to the scoring method disclosed by WOOLFE (Exper. Chemother., Acad. Press, New-York-London, 1963, p. 422—443): the average infestation index varies from 0 to 5.

Some of the resultats obtained are summarized in Table 3.

TABLE 3.

Product	Daily dosage (mg/kg)	Mean infestation index
RC19	200	0.5
RC61	100	1.2
RC284	100	0.3
none		3.5

Acute toxicity was determined in SWISS SPF mice by individual forcible feeding in the form of a homogeneous suspension, in a single administration. The following results were obtained after 14 days:

RC12 LD50 : 1.7 g/kg
RC19 LD50 : in excess of 3 g/kg
RC30 LD50 : 2.1 g/kg
RC284 LD50 : 2.2 g/kg

The antibacterial and antifungal activities were evaluated by the determination of the minimum inhibitory concentration, as mcg/cm3, of compounds (I) with respect to various pathogenic microbial strains. Two antifungal antibiotics, nystatine and griseofulvine, are used as reference materials.

The results obtained are given in Table 4 below.

TABLE 4

Minimum inhibitory concentration (as mcg/cm3)

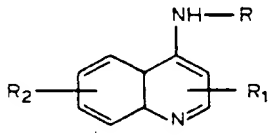
Product	1	2	3	4	5	
RC 61	2	4	1	0.8	0.5	
RC 2	2	2	<1	<0.25	0.25	
RC 3	6	6	6	2	<1	
RC 410	20	20	2.5	0.8	1	
RC 57	6	6	1.5	0.5	1	
RC 14	2	2	2	0.4	0.4	
RC 17	2	2	2	0.4	0.4	
RC 31	2	4	2	0.5	0.5	
RC 46	2	2	2	0.5	0.5	
RC 24	6	6	3	<0.25	0.8	5000 U/mg
Nystatine			15 U			
Griseofulvine				0.8	0.8	

1 : *Staphylococcus aureus* 209P—ATCC 6538P
2 : *Streptococcus fecalis*
3 : *Candida albicans*
4 : *Trichophyton mentagrophytes*
5 : *Epidermophyton floccosum*

In these various applications, the compounds (I) may be administered orally, topically, or by the vaginal or rectal route, formulated as tablets, cachets, capsules, ointments, solutions, powders, mouth wash, ovules, or suppositories, optionally together with the excipients conventionally used in such formulations. A daily dosage regimen of 0.5—5 g active ingredient may generally be administered.

WHAT WE CLAIM IS:—

1. 4-amino-quinoline derivatives having the general formula:



(I)

in which R is a straight- or branched-chain alkyl group having at least 10 carbon atoms and R_1 and R_2, which may be the same or different, are each hydrogen, halogen, an alkyl, aryl, hydroxy, ether, thioether, amino, alkylamino, dialkylamino, nitro or trifluoromethyl group, and their acid addition salts.

2. Derivatives as claimed in claim 1, wherein R_1 and R_2 are each hydrogen, halogen, a lower alkyl, phenyl, hydroxy, lower alkoxy, lower alkylthio, diloweralkylamino, nitro or trifluoromethyl group.

3. 4-n-Dodecylamino-quinoline and its salts.

4. 8-Isopropylamino-4-n-decylamino-quinaldine and its salts.

5. 7-Methyl-4-n-decylamino-quinoline and its salts.

6. 7-Chloro-4-n-decylamino-quinoline and its salts.

7. 6-Methoxy-4-n-decylamino-quinoline and its salts.

8. 8-Methoxy-4-n-decylamino-4-quinaldine and its salts.

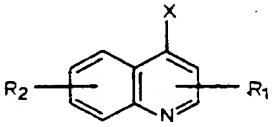
9. 6-Nitro-4-n-decylamino-quinoline and its salts.

10. 7-Nitro-4-n-decylamino-quinoline and its salts.

11. 4-n-Decylamino-quinoline and its salts.

12. 8-Methyl-4-n-decylamino-quinaldine and its salts.

13. Process for the preparation of derivatives as claimed in any one of the preceding claims, comprising reacting a quinoline having the formula:



(II)

in which R_1 and R_2 have the aforesaid meanings and X is a cleavable grouping, with a primary amine of the formula NH_2R in which R has the aforesaid meaning, and, if desired, converting the resulting compound to the salt form, by means of an acid.

14. Process as claimed in claim 13, wherein X is halogen, a hydroxy, aryloxy, alkylthio or arylthio, alkyl- or aryl-sulfonyl group.

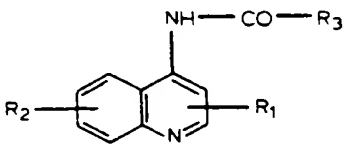
15. Process as claimed in claim 13 or 14, wherein there is used, per mole of quinoline (II), either a substantially dimolar amount of amine NH_2R, or a substantially equimolar amount of said amine, to which is added a tertiary amine.

16. Process as claimed in any one of claims 13 to 15, wherein the reaction is conducted at elevated temperature, in the presence of a solvent.

17. Process as claimed in claim 16 wherein the solvent is an alcohol, a nitro derivative, acetonitrile, phenol or a mixture thereof.

18. Process as claimed in any one of claims 13 to 16, wherein after compound (I) is obtained, it is converted to another compound (I) by chemical modification of substituents R_1 and/or R_2.

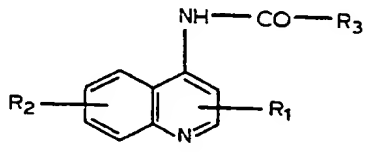
19. Process for the preparation of derivatives as claimed in any one of claims 1 to 12, comprising reducing an amide having the formula:



(III)

in which R_1 and R_2 have the aforesaid meanings and R_3 is an alkyl group having at least 9 carbon atoms, to an amine (I), with lithium aluminum hydride in a solvent, and, if desired, converting the resulting amine to a salt by means of an acid.

20. Process for the preparation of derivatives as claimed in any one of claims 1—12, comprising oxidizing an amide having the formula:

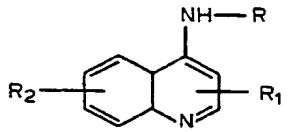


(III)

in which R_1 and R_2 have the aforesaid meanings and R_3 is an alkyl group having at least 10 carbon atoms, to an amine (I), with a hypochlorite or a hypobromite and, if desired, converting the resulting amine to a salt by means of an acid.

21. Therapeutic composition comprising, as active ingredient, a compound as claimed in any one of claims 1—12.

22. 4-amino-quinoline derivatives, having the general formula:



(I)

in which R, R_1 and R_2 have the same significance as in claim 1 and their acid addition salts, substantially as described with reference to the Examples.

23. Process for the preparation of derivatives as claimed in claim 22, substantially as described with reference to the Examples.

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$N^4,N^{4'}$ - DECAMETHYLENE - BIS(4 - AMINO) - QUINALDINE DIACETATE COMPOSITIONS FOR CONTROLLING TOPICAL INFECTIONS

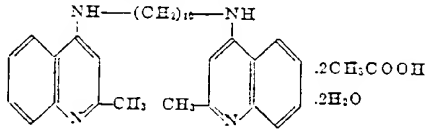
Robert R. Strauss, Cheltenham, and Paul D. Rosenstock, Philadelphia, Pa., assignors to Richardson-Merrell Inc., New York, N.Y., a corporation of Delaware

No Drawing. Filed June 4, 1965, Ser. No. 461,531

2 Claims. (Cl. 167—58)

This invention relates to new compositions of matter useful for controlling topical infections by bacteria, fungi, and other microorganisms. The invention includes the active compound, methods of preparing the same, compositions of matter containing the active compound, and methods of using these new compositions in the control of topical infections.

The new compound of this invention is the water soluble diacetate salt of $N^4,N^{4'}$-decamethylene-bis-(4-amino-quinaldine). It is usually obtained in the form of a dihydrate. More particularly the compound of this invention has the following structure:



This diacetate-dihydrate salt of $N^4,N^{4'}$-decamethylene-bis-(4-aminoquinaldine) shows high antimicrobial activity with low topical toxicity. In contrast to the high order of activity against *Pseudomonas aeruginosa* shown by this salt, the free base of this compound (a known compound) is relatively inactive having only one-fourth the activity of the above salt. It is well known that *Pseudomonas aeruginosa* is refractory to most chemotherapeutic agents.

It is an object of this invention, therefore, to provide a broad spectrum antimicrobial agent effective against both gram positive and gram negative bacteria, filamentous and yeast-like fungi and the parasitic protozoan *Trichomonas vaginalis*. In vitro activity is not adversely effected in the presence of 10 percent normal serum. Compositions of matter useful in topical treatment of skin, mouth, nose, ear, and other infections caused by a wide variety of pathogens are also included within the scope of the invention.

The in vitro antibacterial spectrum of $N^4,N^{4'}$-decamethylene-bis-(4-aminoquinaldine) diacetate - dihydrate was determined against bacteria and fungi in the presence and absence of 10 percent normal horse serum. The compound was tested against the organisms listed in the following table by two fold serial tube dilutions in Trypticase soya broth growth medium and incubation of the organisms for twenty-four hours at 37° C. The lowest concentration of N^4,N^4 - decamethylene-bis-(4-amino-quinaldine) diacetate which prevented visible growth after twenty-four hours of incubation at 37° C. was designated as the minimal inhibitory concentration. The results are shown in the table which follows.

ANTIBACTERIAL SPECTRA (IN VITRO)

[Minimum inhibitory concentration (mcg./ml.)]

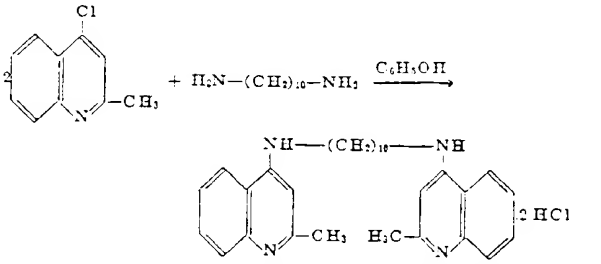
Test Organism	$N^4,N^{4'}$-Decamethylene-bis-(4-Aminoquinaldine) Diacetate-Dihydrate	
	Alone	+ Serum
Staphylococcus aureus	6.3	12.5
Streptococcus pyogenes	1.6	6.3
Escherichia coli	100.0	25.0
Pseudomonas aeruginosa	12.5	25.0
Candida albicans	10.0	10.0
Trichophyton mentagrophytes	25.0	10.0
Trichomonas vaginalis	10.0	10.0

In vivo studies with *Trichomonas vaginalis* were done in 18 to 20 gram white mice (ten per group) which were infected subcutaneously daily for three days with the organism. Each infection was followed two hours later by treatment with $N^4,N^{4'}$-decamethylene-bis-(4-amino-quinaldine) diacetate-dihydrate by the same route at doses of 1.0, 0.1, and 0.01 milligram per mouse in 0.5 milliliter of solution. Five days after the last infection-treatment regime, the animals were sacrificed and the infection sites were washed out with sterile saline and examined for viable trichomonads microscopically and by culture in simplified Trypticase soya serum base medium. The results showed that 10 mcg./mouse, the in vitro minimum inhibitory concentration, was sufficient to protect all ten animals in the test groups.

Also it has been found that unlike other topically useful anti-microbial agents, N^4,N^4 - decamethylene-bis-(4-aminoquinaldine) diacetate-dihydrate is not inactivated in the presence of sebum. The compound was tested in the agar cup plate zone inhibition test with 0.25 percent synthetic sebum emulsified in Eugon agar, and without synthetic sebum. The results showed that the size of the zone of inhibition was the same in the synthetic medium containing sebum as in the medium in which this material was not present, thus indicating that the synthetic sebum had no apparent effect on the activity of the salt. The microorganism used was *Staphylococcus aureus* 209.

In another in vitro experiment in which *Staphylococcus aureus* 209 was the microorganism, the number of viable bacteria per milliliter remained substantially constant in both the control and in the medium containing 0.25 percent synthetic sebum, but when 500 micrograms per milliliter of the water soluble salt of the present invention was added, the viable bacteria population decreased to zero (0) in sixty minutes under the same conditions. Surprisingly, in the medium containing both the salt of the present invention and 0.25 percent synthetic sebum, there were no viable bacteria found in the medium at the end of thirty minutes. Apparently, the presence of the sebum favorably influenced the antibacterial activity of the salt.

The compound of this invention may be prepared by the reaction of 1,10-diaminodecane with 4-chloroquinaldine in the presence of phenol at 150° C., i.e.



which gives the dihydrochloride salt. Upon liberation of the free base, the product is converted to the diacetate-dihydrate salt in methanol-ether solution.

EXAMPLE 1

$N^4,N^{4'}$-decamethylene-bis-(4-aminoquinaldine) diacetate dihydrate

In a five liter flask equipped with a stirrer, dropping funnel, and thermometer was placed 145 grams (1 mole) of 1,10-diaminodecane and 900 grams of phenol. The mixture was heated until the temperature of the melt reached 115° C., external heat was removed, and 300 grams (2.01 mole) of 4-chloroquinaldine was added at such a rate that the temperature did not rise above 150° C. After the addition of the 4-chloroquinaldine was completed, the reaction mixture was heated for an additional hour at 145° C. and

then allowed to cool to room temperature. Upon addition of 1.2 liters of dry ether a solid precipitated. The solid was filtered and washed with ether to give the desired N^4,$N^{4'}$-decamethylene-bis-(4-aminoquinaldine) as the dihydrochloride salt melting at 120° C. to 126° C.

The hydrochloride salt was dissolved in 5 liters of hot methanol and the solution made alkaline by addition of 400 milliliters of 50 percent sodium hydroxide solution. Upon cooling, a solid separated, was filtered and washed with water, acetone and ether to give the desired N^4,$N^{4'}$-decamethylene-bis-(4-aminoquinaldine) melting at 181° C. to 185° C.

To a suspension of the free base, N^4,$N^{4'}$-decamethylene-bis-(4-aminoquinaldine), in 600 milliliters of methanol was added 80 milliliters of glacial acetic acid. The resulting solution was stirred and 2 liters of anhydrous ether added. Stirring was continued while the solution was cooled in an ice bath causing the separation of a solid. The solid was filtered, washed with ether and allowed to air dry to give a solid melting at 120° C. to 124° C. The product was then dissolved in hot methanol and decolorized with 1 gram of charcoal. After filtering, the solution was diluted with ether and the resulting solid further purified by an additional crystallization from methanol-ether to give the desired N^4,$N^{4'}$-decamethylene-bis-(4 - aminoquinaldine) diacetate dihydrate as an off-white solid melting at 125° C. to 130° C.

EXAMPLE 2

Five percent (5%) ointment

One hundred and twenty grams of glyceryl monostearate, 100 grams of white petrolatum, 25 grams of stearyl alcohol, 35 grams of a mixture of wool fat alcohols, and 10 grams of sorbitan monosterate are melted together. Separately, 5 grams of methylparaben, 100 grams of propylene glycol, 25 grams of polyoxyl 40 stearate, and 50 grams of N^4,$N^{4'}$-decamethylene-bis-(4-aminoquinaldine) diacetate dihydrate are dissolved in 530 milliliters of purified water and heated to 70° C. to 80° C. The aqueous phase is filtered and added to the oil phase at 70° C. to 80° C. with stirring. Sufficient purified water is added to the ointment to make a total of 1 kilogram.

EXAMPLE 3

One-tenth percent (0.1%) ointment

One hundred and twenty grams of glyceryl monostearate, 100 grams of white petrolatum, 25 grams of stearyl alcohol, 35 grams of a mixture of wool fat alcohols, and 10 grams of sorbitan monostearate are melted together. Separately, 5 grams of methylparaben, 100 grams of propylene glycol, and 25 grams of polyoxyl 40 stearate are dissolved in 530 milliliters of purified water and heated to 70° C. to 80° C. The aqueous phase is added to the oil phase at 70° C. to 80° C. with stirring. One gram of N^4, $N^{4'}$-decamethylene-bis-(4-aminoquinaldine) diacetate dihydrate is dissolved in 50 milliliters of purified water and heated to approximately 60° C. The solution is then filtered and added to the ointment with stirring. Sufficient purified water is added to the ointment to make a total of 1 kilogram.

EXAMPLE 4

Five-tenths percent (0.5%) lotion

Fifty grams of mineral oil and 30 grams of cetyl alcohol are heated to 70° C. to 80° C. Separately, 1.25 grams of propylparaben, 30 grams of polyoxyl 40 stearate, 10 grams of glycerin, 5.0 grams of N^4,$N^{4'}$-decamethylene-bis-(4-aminoquinaldine) diacetate dihydrate, and 550 milliliters of purified water are heated to approximately 75° C. and the solution is filtered. The aqueous phase is added to the oil phase with efficient mixing until the preparation has cooled.

EXAMPLE 5

One percent (1%) oral wash or spray

Three hundred milligrams of methylparaben and 200 milligrams of propylparaben are dissolved in 30 milliliters of alcohol. Ten grams of N^4,$N^{4'}$-decamethylene-bis-(4-aminoquinaldine) diacetate dihydrate is dissolved in a mixture of 200 milliliters of 0.1 M lactic acid-sodium lactate buffer solution of pH 5.5, 200 milliliters of glycerin, and 100 milliliters of purified water. The parabens solution is then added. A suitable flavor and certified F.D. and C. dye are added, followed by sufficient purified water to make a total volume of 1 liter. The solution is then filtered.

What is claimed is:

1. An antimicrobial composition for the control of topical infections by pathogenic microorganisms which comprises N^4,$N^{4'}$-decamethylene-bis-(4-aminoquinaldine) diacetate dihydrate in a carrier selected from the class consisting of ointments, lotions, oral washes and sprays which may be safely applied to the skin.

2. A method of controlling topical infections in animals which are caused by microorganisms which comprises applying to the infected site the compositions of claim 1.

References Cited

UNITED STATES PATENTS

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ALBERT T. MEYERS, *Primary Examiner.*

S. ROSEN, *Examiner.*

D. R. MAHANAND, *Assistant Examiner.*

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$N^4,N^{4'}$ - DECAMETHYLENE - BIS(4 - AMINO) - QUINALDINE DIACETATE COMPOSITIONS FOR CONTROLLING TOPICAL INFECTIONS

Robert R. Strauss, Cheltenham, and Paul D. Rosenstock, Philadelphia, Pa., assignors to Richardson-Merrell Inc., New York, N.Y., a corporation of Delaware

No Drawing. Filed June 4, 1965, Ser. No. 461,531
2 Claims. (Cl. 167—58)

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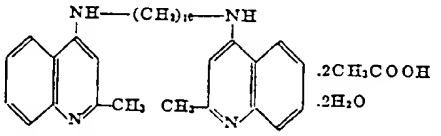

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It is an object of this invention, therefore, to provide a broad spectrum antimicrobial agent effective against both gram positive and gram negative bacteria, filamentous and yeast-like fungi and the parasitic protozoan *Trichomonas vaginalis*. In vitro activity is not adversely effected in the presence of 10 percent normal serum. Compositions of matter useful in topical treatment of skin, mouth, nose, ear, and other infections caused by a wide variety of pathogens are also included within the scope of the invention.

The in vitro antibacterial spectrum of $N^4,N^{4'}$-decamethylene-bis-(4-aminoquinaldine) diacetate - dihydrate was determined against bacteria and fungi in the presence and absence of 10 percent normal horse serum. The compound was tested against the organisms listed in the following table by two fold serial tube dilutions in Trypticase soya broth growth medium and incubation of the organisms for twenty-four hours at 37° C. The lowest concentration of N^4,N^4 - decamethylene-bis-(4-aminoquinaldine) diacetate which prevented visible growth after twenty-four hours of incubation at 37° C. was designated as the minimal inhibitory concentration. The results are shown in the table which follows.

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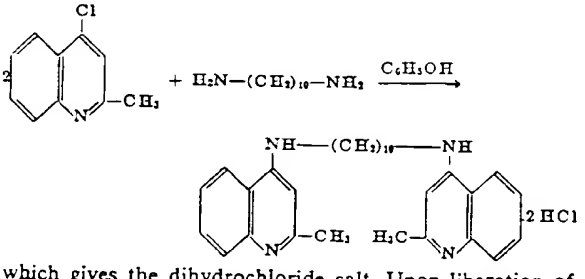

which gives the dihydrochloride salt. Upon liberation of the free base, the product is converted to the diacetate-dihydrate salt in methanol-ether solution.

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One-tenth percent (0.1%) ointment

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